MAJOR ARTICLE

A West Nile Virus DNA Vaccine Utilizing a Modified Promoter Induces Neutralizing Antibody in Younger and Older Healthy Adults in a Phase I Clinical Trial

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Background. West Nile virus (WNV) is a flavivirus that causes meningitis and encephalitis. There are no licensed vaccines to prevent WNV in humans. The safety and immunogenicity of a first-generation WNV DNA vaccine was demonstrated in a clinical trial and a similar DNA vaccine has been licensed for use in horses.

Methods. A DNA vaccine encoding the protein premembrane and the E glycoproteins of the NY99 strain of WNV under the transcriptional control of the CMV/R promoter was evaluated in an open-label study in 30 healthy adults. Half of the subjects were age 18–50 years and half were age 51–65 years. Immune responses were assessed by enzyme-linked immunosorbent assay, neutralization assays, intracellular cytokine staining, and ELISpot.

Results. The 3-dose vaccine regimen was safe and well tolerated. Vaccine-induced T cell and neutralizing antibody responses were detected in the majority of subjects. The antibody responses seen in the older age group were of similar frequency, magnitude, and duration as those seen in the younger cohort.

Conclusions. Neutralizing antibody responses to WNV were elicited by DNA vaccination in humans, including in older individuals, where responses to traditional vaccine approaches are often diminished. This DNA vaccine elicited T cell responses of greater magnitude when compared with an earlier-generation construct utilizing a CMV promoter.

Clinical Trials Registration. NCT00300417.

West Nile virus (WNV) is a flavivirus transmitted primarily by mosquitoes to a variety of vertebrate hosts. Flaviviruses are positive-stranded RNA viruses and include important human pathogens such as yellow fever virus, St Louis encephalitis virus, dengue virus, and Japanese encephalitis virus (JEV). WNV was initially isolated from a human residing in the West Nile district of Uganda in 1937 [1]. The virus is present throughout Africa, Asia, the Middle East, and the Americas. WNV was first recognized in the United States in 1999 when it caused an epidemic in New York state. Since 1999, WNV has spread throughout the Americas [2–4]. The incidence in the United States peaked at 9862 cases in 2003. The infection is now considered endemic in the United States and in 2009 there were 720 reported cases [5, 6].

The mature WNV virion is composed of 180 copies of the envelope protein (E) arranged with pseudo T = 3 icosahedral symmetry. The nucleocapsid core contains copies of RNA encoding for genome and capsid

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proteins, and the general arrangement of WNV is similar to that of dengue virus [7]. The major surface protein (E) mediates interactions with the cell surface and facilitates fusion between the virus and cell membranes. Virions also incorporate the protein premembrane (prM), which is cleaved into a smaller virion-associated membrane (M) peptide during virion maturation. Surface envelope proteins are the primary target for the humoral response against flavivirus infection.

WNV is an enzootic infection and is maintained in a mosquito–bird transmission cycle; incidental hosts have been identified, including humans, horses, and alligators [3, 8]. The principal form of transmission to humans is from the bite of a mosquito. Person-to-person transmission has been recognized, including blood transfusion, organ transplantation, breastfeeding, and transplacental or laboratory acquisition [2, 9]. Human illness peaks in late summer or early autumn, reflecting peak viral amplification within the bird–mosquito–bird cycle [1].

WNV infection of humans has been associated with a variety of symptoms from asymptomatic to severe encephalitis. Central nervous system involvement occurs in 1 in 150 patients [10, 11]. Care is supportive but intravenous immunoglobulin, alpha interferon, and ribavirin have been investigated for severe cases [12, 13]. One investigational therapy with potential for benefit is a humanized monoclonal antibody, Hu-E16, which binds to the envelope protein of WNV and has shown efficacy in preclinical testing and safety in clinical testing [14–16].

As vaccines are developed, consideration for those at greatest risk is a priority. For WNV, advanced age is a risk factor for severe disease [17]; however, the mechanism for increased susceptibility in the elderly and immunocompromised remains unknown. Published data suggest a role for antibody in protection and clearance of flavivirus infections [18, 19]. In vitro data also implicate dysregulation of toll-like receptor 3 (TLR3) in macrophages in the elderly, leading to higher cytokine (interleukin [IL]-6, interferon [IFN]-β, tumor necrosis factor [TNF]-α) levels, which are associated with higher viral burdens in macrophages and facilitation of WNV entry into the cerebrospinal fluid secondary to blood-brain barrier disruption. In contrast, in young adults, TLR3 expression declines during WNV infection, diminishing WNV entry and cytokine release [20]. In general, vaccines induce decreased immunity in the elderly [21–23]. Taken together, these data describe immunosenescence, an age-related change in immunity, which may impact the predilection of the aged to become seriously affected by WNV and is a possible reason for the generalized decreased vaccine efficacy seen in older adults [21, 23].

WNV infection is a veterinary health concern, and infection in horses carries a 30%–40% mortality rate [24, 25]. Equine vaccine development provides an animal model for the development of a human WNV vaccine. The equine DNA vaccine, pCBWN (Fort Dodge Animal Healthwith the Centers for Disease Control and Prevention), encodes for the prM and E proteins from WNV in a similar configuration as the DNA vaccine described here. It elicits neutralizing antibody and protects mice and horses from WNV [26]. That vaccine was licensed by the US Department of Agriculture for horses in 2005, and represents the first license issued for a veterinary DNA vaccine [24].

Investigational WNV vaccines for humans have been evaluated in preclinical and clinical studies, and candidate platforms include gene-based vaccines and viral-like particles [27]. A candidate DNA vaccine for WNV has previously been evaluated in a phase I clinical trial (VRC 302) and was shown to be safe and immunogenic. That study provided evidence that a DNA vaccine, based on the equine vaccine, elicited neutralizing antibody in humans [28].

In the current study (VRC 303), a nearly identical recombinant DNA vaccine encoding WNV prM and E proteins was used. This newer-generation DNA plasmid construct differs from the previously tested vaccine construct in that a modified promoter, CMV/R, was utilized rather than the original CMV promoter. The CMV/R promoter includes the regulatory R region from the 5′ long terminal repeat of human T cell leukemia virus type (HTLV-1), which serves as a transcriptional and posttranscriptional enhancer. The CMV/R promoter has improved protein expression of transduced genes, which has been associated with greater immunogenicity following DNA immunization of animals [29]. The CMV/R promoter has been utilized in vaccines in other phase I and II clinical trials [30–32], and although a direct comparison of these promoters in DNA vaccines encoding identical antigens has not been conducted in a randomized clinical trial, the CMV/R promoter has been shown to enhance the immunogenicity of DNA vaccines in both mice and nonhuman primates [29]. The results of the clinical trial reported here allow for a direct comparison of the safety and immunogenicity of a DNA vaccine in 2 age groups (VRC 303) as well as an indirect comparison of this newer-generation WNV vaccine encoding the CMV/R promoter (VRC 303) to the previously published clinical study (VRC 302) [28] results assessing an earlier-generation WNV DNA vaccine utilizing the CMV promoter.

MATERIALS AND METHODS

Study Design

Protocol VRC 303 was a single-site, phase I, open-label study to examine the safety, tolerability, and immune response to an investigational recombinant DNA WNV vaccine. Healthy adult subjects in 2 age groups (18–50 years and 51–65 years) who were negative for WNV immunoglobulin G (IgG) by a commercial assay (Focus Technologies) at baseline were enrolled at the Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health

(NIH), Bethesda, Maryland. Experimental guidelines of the US Department of Health and Human Services were followed in the conduct of clinical research, and the protocol was approved by the NIAID Institutional Review Board. Thirty subjects were enrolled between 14 March 2006 and 16 October 2006.

Vaccine was administered at a 4-mg dose via intramuscular injection in the lateral deltoid using the Biojector 2000® Needle-Free Injection Management System™ (Bioject). Vaccine was administered on study days 0, 28, and 56. Solicited local reactogenicity, systemic reactogenicity, and other nonsolicited adverse events were evaluated by laboratory and clinical evaluations at scheduled study visits and intermittently as needed. Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA), and severity of adverse events was graded using the Division of AIDS Table for Grading the Severity of Adverse Events (NIH, version 1.0). Local and systemic solicited reactogenicity, including pain, erythema, swelling, myalgia, malaise, headache, chills, nausea, and temperature, was collected by subject self-report on 5-day diary cards following each injection. Subjects were followed for a total of 52 weeks and study subject visits were completed in October 2007.

Vaccine

The vaccine VRC - WNVDNA020-00-VP is composed of a single, closed, circular plasmid DNA macromolecule (VCL-8111) constructed to produce the prM and E proteins of the WNV envelope glycoprotein. The plasmid was based on an analogous construct shown to protect mice and horses from virus challenge [26] and on a construct previously shown to be immunogenic in humans [28]. The vaccine plasmid was made by Vical under Good Manufacturing Practices and encodes for a single polypeptide encompassing a modified signal sequence from JEV fused upstream of WNV prM and E coding sequences cloned into the expression vector VR-1012 (CMV/R backbone). The only difference between the vaccine reported in this trial and the vaccine previously assessed in phase I clinical testing (VRC 302) is that the earlier-generation construct, VRC-WNVDNA017-00-VP [28], included the cytomegalovirus immediate early 1 gene promoter (CMV promoter) whereas VRC-WNVDNA020-00-VP includes a modified version of this promoter (CMV/R promoter). The CMV/R promoter contains a regulatory sequence for the R region of the long terminal repeat from the HTLV-1, which enhances transcription and posttranscriptional events [29]. The WNV prM and E sequences are derived from the NY99 human WNV isolate. In vitro expression results in the formation of noninfectious subviral particles (SVPs). As with the previously reported construct, the plasmid in this vaccine is incapable of replication in animal cells and does not permit the generation of an infectious virion even if recombination or gene duplication were to occur.

This DNA vaccine was produced in bacterial cell cultures containing kanamycin selection medium. The process involved *Escherichia coli* fermentation, purification, and formulation as a sterile liquid. The vaccine was manufactured at a 4-mg dose in phosphate-buffered saline.

Antibody Responses by Enzyme-Linked Immunosorbent Assay (ELISA)

Duplicate wells of serial dilutions of volunteer sera were incubated for 1 hour at 37°C on WNV recombinant antigen-coated plates (Focus Technologies) as previously described [28]. Endpoint titers for each volunteer were established as the last dilution with a preimmunization corrected optical density >0.2.

Antibody-Mediated Neutralization Using Reporter Virus Particles

WNV reporter virus particles (RVPs) composed of the structural proteins of the NY99 strain of WNV and a subgenomic replicon were produced by complementation in BHK-21 cells as previously described [33]. Antibody-mediated neutralization was measured using a Raji B-lymphoblastoid cell line that expresses the WNV attachment factor CD209L (DC-SIGNR) as described previously [34]. WNV RVPs were incubated with serial 3-fold dilutions of volunteer sera at room temperature for 2 hours and then added to 5×10^4 cells plated on the day of the assay. Infectivity was monitored 2 days postinfection by flow cytometry. The effective concentration measured as the reciprocal dilution of sera required to neutralize half of the infection events (EC_{50}) was calculated by nonlinear regression as previously described [28]. Data are presented as corresponding to the EC_{50}, and are adjusted to consider the final 300-μL volume of the neutralization reaction.

T cell Responses by ELISpot

ELISpot was performed on subject samples at baseline and after vaccination as previously described [31]. Cells were stimulated overnight with vaccine insert–specific peptide pools (WNV-E and WNV-M) at 2×10^5 cells per well. Results are expressed as mean spot-forming cells per million peripheral blood mononuclear cells.

T cell Responses by Intracelluar Cytokine Staining

CD4 and CD8 T cell responses were measured by intracellular cytokine staining (ICS) at baseline and after vaccination as previously described [31]. Cells were stimulated with vaccine insert–specific peptide pools (WNV-E and WNV-M). Cells were permabilized, washed, and stained with directly conjugated anti-human CD3, CD4, CD8, IFN-γ, and IL-2 antibodies and were assessed for CD3, CD8, CD4, and IFN-γ/IL-2 expression on a FACSCalibur flow cytometer (BD Biosciences).

Statistical Methods

All assays are treated as binary (responders/nonresponders). We use the usual 95% central exact confidence intervals for binomial rates. We are 97.5% confident that the true response rates in the

antibody assays are larger than the lower limit. Calculations were done in R version 2.3.1. A positive T cell response for ICS and ELISpot data was based on composite criteria as previously described in 4 published studies of candidate vaccines [30, 31, 35, 36]. SAS (version 9.0; SAS Institute) and S-plus software (version 6.2; Insightful) were used for analyses.

RESULTS

Study Population

A total of 30 healthy adult subjects were enrolled. Table 1 includes demographic data regarding gender, age, race/ethnicity, body mass index (BMI), and educational level at the time of enrollment. The overall subject population was 60% male, with a mean age of 44 years. Subjects in group 1 ranged in age from 22 to 45 years (mean, 31.5; SD, 8.3) and were 67% male. Subjects in group 2 ranged in age from 51 to 65 years (mean, 56 years; SD, 4.5). Subjects in both groups were predominantly Caucasian (90%) and non-Hispanic/Latino (96.7%). The mean BMI in the younger age group was 25.4 (SD, 4.3) and 27.2 (SD, 4.4) in the older age group. All subjects had an educational level of high school or higher and 93% reported having college or advanced degrees.

Safety

The vaccine was well tolerated and there were no vaccine-related serious adverse events. Local and systemic reactogenicity (Tables 2 and 3) was generally mild and was similar in each of the groups and also similar to that seen in previous DNA vaccine studies of other constructs [28, 30, 31, 37]. Across all subjects, the worst severity of local reactogenicity after any vaccination was none in 10%, mild in 83.3%, and moderate in 6.7%, while the worst severity of systemic symptoms after any vaccination was none in 56.7%, mild in 36.7%, and moderate in 3.3%. The most common local symptom was mild pain and the most common systemic symptoms were mild headache, malaise, and myalgia. Two subjects did not complete the vaccination schedule. One subject in the younger age group (Subject D) withdrew from the study after receiving 1 vaccination due to work schedule demands; immune assessment data are not available for this participant. One subject in the older age group (Subject BB) was withdrawn from the vaccination schedule after beginning a concomitant medication that was an exclusion from further vaccinations, but remained on the study for safety and immunogenicity evaluations.

Antibody Responses

Vaccine-induced humoral immune responses were assessed at weeks 0, 8, 12, and 32. All subjects were negative for WNV antibody by ELISA at baseline, and 1 subject in the 51–65 year age group, Subject T, exhibited a positive neutralizing antibody response (low titer) at baseline that was boosted by vaccination. The primary immune assessment time point was 4 weeks after the final vaccination (week 12 of the study). In text and graphical representation, Subjects 1–30 are represented by an identifier based on age (designated by letters A–DD). Vaccine-induced antibody, assessed by ELISA, was present in 29 of 29 subjects for at least 1 time point, and at week 12 was present in 27 of 29 subjects (Figure 1A). For each vaccinee, a profile of the neutralization activity in sera at each collection time point was measured using 8 dilutions of sera. Neutralizing antibody was demonstrated in 28 of 29 subjects at week 12, including in Subject BB, who received only 1 vaccination (Figure 1B). Antibody responses peaked at week 12 (4 weeks after the final injection) and diminished slightly over the course of the

Table 1. VRC 303 Demographic Characteristics

Characteristic	Category	Ages 18–50 (n = 15)	Ages 51–65 (n = 15) No. (%) of Subjects	Overall (n = 30)
Gender	Male	10 (66.7)	8 (53.3)	18 (60.0)
	Female	5 (33.3)	7 (46.7)	12 (40.0)
Age	Mean (SD)	31.5 (8.3)	56.3 (4.5)	43.9 (14)
	Range	[22, 45]	[51, 65]	[22, 65]
Race	Asian	1 (6.7)	0 (.0)	1 (3.3)
	Black or African American	2 (13.3)	0 (.0)	2 (6.7)
	White	12 (80.0)	15 (100.0)	27 (90.0)
	All other races combined	0 (.0)	0 (.0)	0 (.0)
Ethnicity	Non-Hispanic/Latino	15 (100.0)	14 (93.3)	29 (96.7)
	Hispanic/Latino	0 (.0)	1 (6.7)	1 (3.3)
Body mass index	Mean (SD)	25.4 (4.3)	27.2 (4.4)	26.3 (4.4)
	Range	[17.8, 33.7]	[19.8, 36.3]	[17.8, 36.3]
Education	Less than high school	0 (.0)	0 (.0)	0 (.0)
	High school or equivalent	1 (6.7)	1 (6.7)	2 (6.7)
	College/university	10 (66.7)	6 (40.0)	16 (53.3)
	Advanced degree	4 (26.7)	8 (53.3)	12 (40.0)

Table 2. Summary of Maximum Local Reactogenicity for Any Injection[a]

Local Symptoms and Intensity	Group 1 Ages 18–50 y (n = 15)	Group 2 Ages 51–65 y (n = 15)	All Subjects (n = 30)
Pain/tenderness			
None	2 (13.3)	1 (6.7)	3 (10.0)
Mild	11 (73.3)	14 (93.3)	25 (83.3)
Moderate	2 (13.3)	0 (.0)	2 (6.7)
Severe	0 (.0)	0 (.0)	0 (.0)
Missing	0 (.0)	0 (.0)	0 (.0)
Swelling			
None	9 (60.0)	8 (53.3)	17 (56.7)
Mild	6 (40.0)	7 (46.7)	13 (43.3)
Moderate	0 (.0)	0 (.0)	0 (.0)
Severe	0 (.0)	0 (.0)	0 (.0)
Missing	0 (.0)	0 (.0)	0 (.0)
Redness			
None	7 (46.7)	6 (40.0)	13 (43.3)
Mild	8 (53.3)	9 (60.0)	17 (56.7)
Moderate	0 (.0)	0 (.0)	0 (.0)
Severe	0 (.0)	0 (.0)	0 (.0)
Missing	0 (.0)	0 (.0)	0 (.0)
Any Local Symptom			
None	2 (13.3)	1 (6.7)	3 (10.0)
Mild	11 (73.3)	14 (93.3)	25 (83.3)
Moderate	2 (13.3)	0 (.0)	2 (6.7)
Severe	0 (.0)	0 (.0)	0 (.0)
Missing	0 (.0)	0 (.0)	0 (.0)

NOTE. [a] The local injection site reactions were recorded by clinicians at 30–45 minutes after injection and were then recorded as self-assessments at home by subjects on a 5-day diary card. Systemic reactions were recorded as self-assessments at home by subjects on a 5-day diary card following each injection.

Table 3. Summary of Maximum Systemic Reactogenicity for Any Injection

Systemic Symptoms and Intensity	Group 1 Ages 18–50 y (n = 15)	Group 2 Ages 51-65 y (n = 15)	All Subjects (n = 30)
Malaise			
None	10 (66.7)	11 (73.3)	21 (70.0)
Mild	4 (26.7)	3 (20.0)	7 (23.3)
Moderate	0 (.0)	1 (6.7)	1 (3.3)
Severe	0 (.0)	0 (.0)	0 (.0)
Missing	1 (6.7)	0 (.0)	1 (3.3)
Myalgia			
None	9 (60.0)	12 (80.0)	21 (70.0)
Mild	5 (33.3)	3 (20.0)	8 (26.7)
Moderate	0 (.0)	0 (.0)	0 (.0)
Severe	0 (.0)	0 (.0)	0 (.0)
Missing	1 (6.7)	0 (.0)	1 (3.3)
Headache			
None	11 (73.3)	11 (73.3)	22 (73.3)
Mild	3 (20.0)	4 (26.7)	7 (23.3)
Moderate	0 (.0)	0 (.0)	0 (.0)
Severe	0 (.0)	0 (.0)	0 (.0)
Missing	1 (6.7)	0 (.0)	1 (3.3)
Chills			
None	13 (86.7)	15 (100.0)	28 (93.3)
Mild	1 (6.7)	0 (.0)	1 (3.3)
Moderate	0 (.0)	0 (.0)	0 (.0)
Severe	0 (.0)	0 (.0)	0 (.0)
Missing	1 (6.7)	0 (.0)	1 (3.3)
Nausea			
None	11 (73.3)	14 (93.3)	25 (83.3)
Mild	3 (20.0)	0 (.0)	3 (10.0)
Moderate	0 (.0)	1 (6.7)	1 (3.3)
Severe	0 (.0)	0 (.0)	0 (.0)
Missing	1 (6.7)	0 (.0)	1 (3.3)
Temperature			
None	14 (93.3)	15 (100.0)	29 (96.7)
Mild	0 (.0)	0 (.0)	0 (.0)
Moderate	0 (.0)	0 (.0)	0 (.0)
Severe	0 (.0)	0 (.0)	0 (.0)
Missing	1 (6.7)	0 (.0)	1 (3.3)
Any Systemic Symptom			
None	9 (60.0)	8 (53.3)	17 (56.7)
Mild	5 (33.3)	6 (40.0)	11 (36.7)
Moderate	0 (.0)	1 (6.7)	1 (3.3)
Severe	0 (.0)	0 (.0)	0 (.0)
Missing	1 (6.7)	0 (.0)	1 (3.3)

NOTE. Data are number (%) of subjects.

yearlong study. The kinetics of the antibody responses was similar in both age groups (Figure 2).

T cell Responses

Vaccine-specific T cell responses were elicited against WNV-E and WNV-M. The peak frequency and magnitude of the responses were detected at week 12. Vaccine-specific T cell responses to WNV-E were detected by ELISpot in 13 of 29 subjects (45%) and to WNV-M in 8 of 29 subjects (28%). CD4 T cell responses to WNV-E, by ICS, were detected in 13 of 29 subjects (45%) and to WNV-M in 1 subject, while CD8 T cell responses to WNV-E were detected in 8 of 29 (28%) and to WNV-M in 7 of 29 (24%) subjects.

Immunogenicity Compared With Previous Phase I Trial

Identical vaccination schedule, dose, route and mechanism of delivery, times of collection (with 1 additional 52-week timepoint), assays, and analysis were conducted in this trial as in the previous trial assessing the WNV DNA vaccine with the CMV promoter in subjects aged 18–50 years [28]. A comparison of the data from VRC 302 shows a significant increase in the frequency and magnitude of T cell responses to the prM protein in the current VRC 303 trial, whereas in the previous VRC 302 trial, T cell immune responses were almost entirely against the E antigen (Figure 3). There is also a significant increase in antigen-specific CD8 T cell responses to WNV-E in the current trial compared

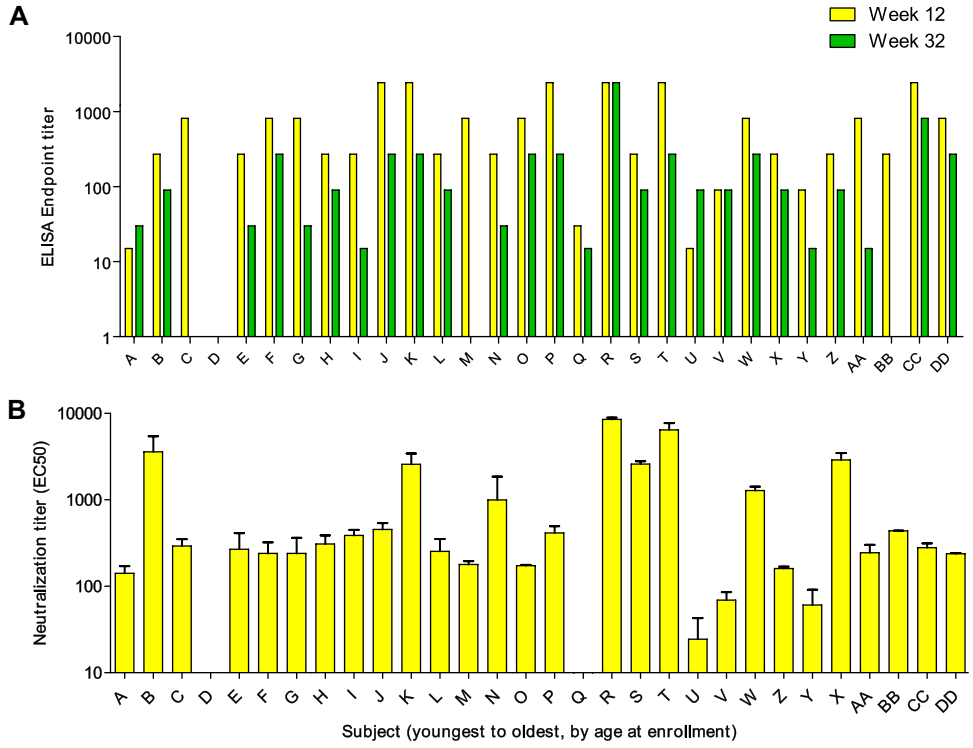

Figure 1. A. Sera from vaccinees at week 12 (4 weeks after 3rd vaccination) and at the final study visit (week 52) was assessed for the presence of antibody by enzyme-linked immunosorbent assay (ELISA) (A) and at week 12 for neutralizing antibody by a West Nile virus reporter virus particle neutralization assay (B). In each figure, the x-axis represents individual vaccine clinical trial subjects A–DD by increasing age at enrollment. The y-axis represents the log10 reciprocal endpoint titer (A) and log10 reciprocal EC_{50} (B).

with the previous trial ($P = .037$); although the response rates are not statistically significantly different for WNV-M, the response rate in the current study is 4 times higher than in the previous trial (Figure 3B). Neutralizing antibody responses trend toward a greater magnitude in the current trial, especially in the older age group, compared with subjects in the previous trial (Figure 4). There is also a trend toward higher antibody responses in the VRC 303 younger group than those seen in VRC 302, which represents an age-matched comparison (18–50 years of age) (Figure 4).

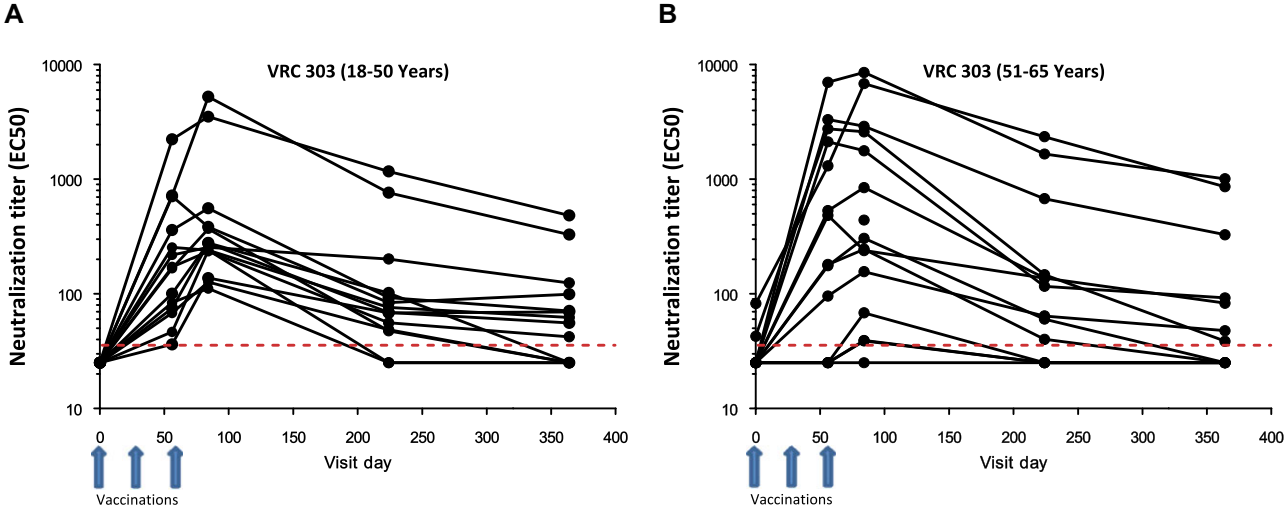

Figure 2. Neutralizing antibody was measured throughout the trial as is shown for the 18–50-year-old subjects (A) and the 51–65-year-old subjects (B). Peak responses occurred around week 12 and responses generally remained positive but at relatively lower magnitude by the end of the yearlong study, Vaccinations were administered at days 0, 28, and 56.

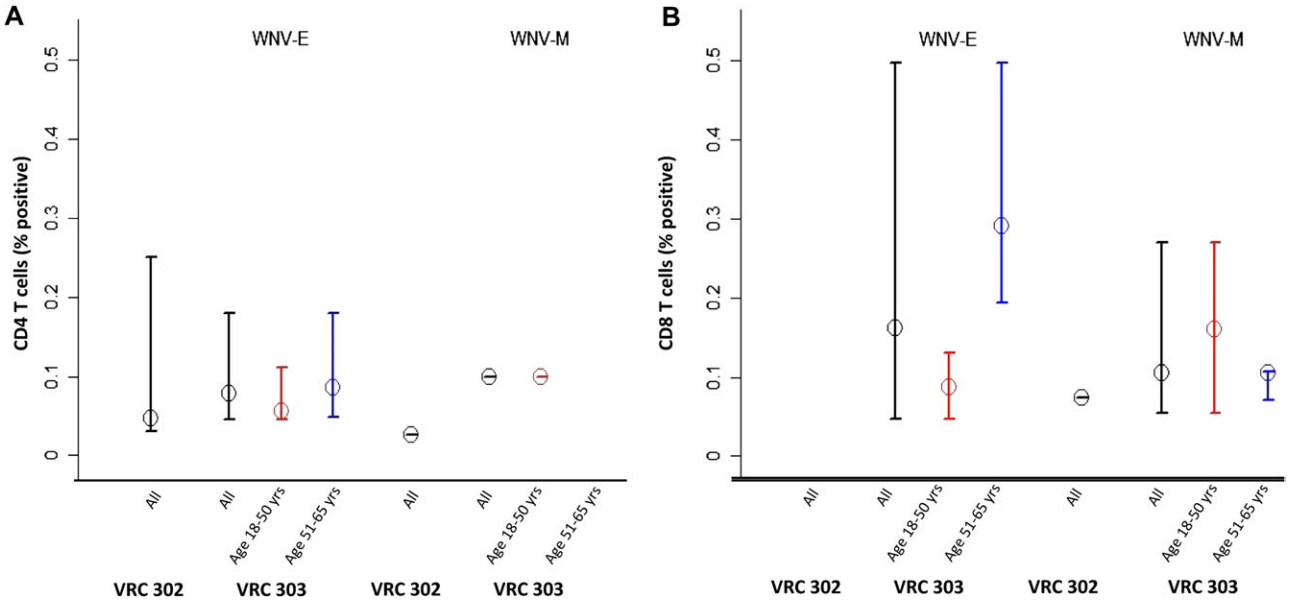

Figure 3. CD4 (A) and CD8 (B) T cell responses over the course of the studies are shown as assessed by intracellular cytokine staining. Magnitude of response is shown for all positive responders by group. Results from the prior study, VRC 302 (all subjects, ages 18–50) are shown in comparison to VRC 303 (all subjects), which are additionally shown by age group, younger (18–50 years) and older (51–65 years).

DISCUSSION

This VRC WNV candidate DNA vaccine utilizing the CMV/R promoter was well tolerated in 30 healthy human subjects. The rate and severity of reactogenicity reported is similar to that reported in the previous WNV candidate DNA vaccine study and in studies of other DNA vaccines [35, 37]. The vaccine was shown to elicit neutralizing antibodies, was more immunogenic than a nearly identical vaccine, and was equally immunogenic in younger and older adults. The previously published phase I trial (VRC 302) of an earlier WNV candidate vaccine appears to be the first report of neutralizing antibody activity elicited by a DNA vaccine in humans [28, 38]. A similar level of neutralizing antibody activity was found to be protective in animal models of infection [26].

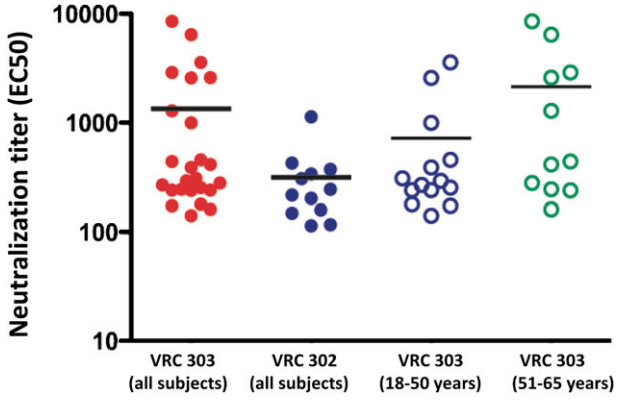

Figure 4. Neutralizing antibody (EC_{50}) responses at week 12 are shown by group: VRC 303 all subjects, VRC 302 all subjects, VRC 303 younger group (ages 18–50 years), and VRC 303 older group (ages 51–65 years).

There is evidence in this study that the improved construct utilizing the CMV/R promoter allowed for enhanced immunogenicity. T cell immune responses in the clinical trial described here were greater in frequency and magnitude and humoral responses trended toward a greater magnitude than in the previous clinical trial. The ability of these WNV DNA vaccines to elicit neutralizing antibody may theoretically be due to the formation of virus-like particles produced by the vaccine-encoded proteins. E and prM are known targets of WNV neutralizing antibody, and SVP formation may allow for relatively authentic antigenic sites constrained by presentation in the icosahedral structure to efficiently induce the relevant antibody specificity [28, 33].

The vector backbone of the original WNV DNA vaccine was modified by enhancing expression of transgenes using a CMV/R promoter. This study evaluating the WNV DNA (CMV/R) vaccine marks the first time that the Vaccine Research Center at NIH has compared identical DNA plasmid vaccines using different promoters, albeit in separate but similar studies. The exact mechanism behind the beneficial effects of the HTLV-1 regulatory region in the modified CMV/R promoter has yet to be determined [29]. We showed enhanced immunogenicity in humans with the WNV DNA vaccine using the CMV/R promoter compared with the wild-type CMV promoter studied in the previous clinical trial [28].

The evaluation of this vaccine in subjects ages 51–65 years is an important step in understanding the issue of immunosenescence

in the context of DNA platform technology. Immunity in response to vaccination is known to diminish as people age, and this can begin at age 45 years [21, 23, 39]. This DNA vaccine induced comparable responses in the 2 age groups and there was a trend toward an improved immune response in the older age group. This finding suggests that the DNA platform may provide a mechanism for effective vaccination in older individuals. This finding was not anticipated based on prior experience in vaccine efficacy studies, which routinely show less immunogenicity in the elderly [21, 23]. Several possibilities exist to explain this observed effect. One possibility is that DNA vaccines may have advantages over other platform technologies in the aging immune system. Older cells may be less resistant to the uptake of random DNA. Alternative explanations involve features of the antigen or delivery approach that simply exceed the threshold of response in both younger and older subjects. For example, the E and prM antigens expressed by the transgene may be inherently more immunogenic in humans. Alternatively, the use of the CMV/R promoter may more efficiently stimulate professional antigen-presenting cells to help promote an antigen-specific T cell response [29]. Finally, the use of the Biojector may aid in the augmentation of responses through effective antigen delivery and this may be enhanced in aged skin. Needle-free immunization has been shown to deposit antigen in a conelike distribution through the stratum corneum into the epidermis and dermis, with minimal deposition in the more superficial layers and the majority of vaccine deposited in the muscle [40]. By utilizing multilayer distribution, antigen is presented directly to a dense network of dendritic cells that migrate to draining lymph nodes, present antigen, and amplify the immune response [41]. The impact on mature skin has not been evaluated and a better understanding may provide insight into the potential of the DNA platform for immunization of the elderly.

DNA vaccines have many favorable features: they are safe, relatively easy to construct, can be produced efficiently, and can induce both T cell and antibody responses. This study showed that the immunogenicity of this DNA vaccine was augmented with the CMV/R promoter, and safety and immunogenicity were preserved in an older population. These data suggest that additional investigation of DNA vaccines is warranted in subjects stratified by age and should include subjects older than age 50.

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References

1. Petersen LR, Marfin AA, Gubler DJ. West Nile virus. JAMA **2003**; 290:524–8.
2. Sampathkumar P. West Nile virus: epidemiology, clinical presentation, diagnosis, and prevention. Mayo Clin Proc **2003**; 78:1137–43;quiz 1144.
3. Hayes EB, Gubler DJ. West Nile virus: epidemiology and clinical features of an emerging epidemic in the United States. Annu Rev Med **2006**; 57:181–94.
4. Morales MB, Barrandeguy M, Fabbri C. West Nile virus isolation from equines in Argentina. Emerg Infect Dis **2006**; 12:1559–61.
5. Murray KO, Mertens E, Despres P. West Nile virus and its emergence in the United States of America. Vet Res **2010**; 41:67.
6. CDC. Final 2009 West Nile virus activity in the United States. http://www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCount09_detailed.htm: Division of Vector Borne Infectious Diseases, 2010.
7. Mukhopadhyay S, Kim BS, Chipman PR, Rossmann MG, Kuhn RJ. Structure of West Nile virus. Science **2003**; 302:248.
8. Jacobson ER, Ginn PE, Troutman JM, et al. West Nile virus infection in farmed American alligators (*Alligator mississippiensis*) in Florida. J Wildl Dis **2005**; 41:96–106.
9. Granwehr BP, Lillibridge KM, Higgs S, et al. West Nile virus: where are we now? Lancet Infect Dis **2004**; 4:547–56.
10. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. Ann Intern Med **2002**; 137:173–9.
11. Sejvar JJ, Hossain J, Saha SK, et al. Long-term neurological and functional outcome in Nipah virus infection. Ann Neurol **2007**; 62:235–42.
12. Ben-Nathan D, Gershoni-Yahalom O, Samina I, et al. Using high titer West Nile intravenous immunoglobulin from selected Israeli donors for treatment of West Nile virus infection. BMC Infect Dis **2009**; 9:18.
13. Makhoul B, Braun E, Herskovitz M, Ramadan R, Hadad S, Norberto K. Hyperimmune gammaglobulin for the treatment of West Nile virus encephalitis. Isr Med Assoc J **2009**; 11:151–3.
14. Oliphant T, Engle M, Nybakken GE, et al. Development of a humanized monoclonal antibody with therapeutic potential against West Nile virus. Nat Med **2005**; 11:522–30.
15. Thompson BS, Moesker B, Smit JM, Wilschut J, Diamond MS, Fremont DH. A therapeutic antibody against West Nile virus neutralizes infection by blocking fusion within endosomes. PLoS Pathog **2009**; 5:e1000453.
16. Beigel JH, Nordstrom JL, Pillemer SR, et al. Safety and pharmacokinetics of single intravenous dose of MGAWN1, a novel monoclonal antibody to West Nile virus. Antimicrob Agents Chemother **2010**; 54:2431–6.
17. Watson JT, Pertel PE, Jones RC, et al. Clinical characteristics and functional outcomes of West Nile fever. Ann Intern Med **2004**; 141:360–5.
18. Diamond MS, Shrestha B, Marri A, Mahan D, Engle M. B cells and antibody play critical roles in the immediate defense of disseminated infection by West Nile encephalitis virus. J Virol **2003**; 77:2578–86.
19. Diamond MS, Shrestha B, Mehlhop E, Sitati E, Engle M. Innate and adaptive immune responses determine protection against disseminated infection by West Nile encephalitis virus. Viral Immunol **2003**; 16:259–78.
20. Kong KDK, Wang X, Qian F, et al. Dysregulation of TLR3 impairs the innate immune response to West Nile virus in the elderly. J Virol **2008**; 82:7613–23.

21. Targonski PV, Jacobson RM, Poland GA. Immunosenescence: role and measurement in influenza vaccine response among the elderly. Vaccine **2007**; 25:3066–9.
22. Andersson CR, Vene S, Insulander M, Lindquist L, Lundkvist A, Gunther G. Vaccine failures after active immunisation against tick-borne encephalitis. Vaccine **2010**; 28:2827–31.
23. Kumar R, Burns E. Age-related decline in immunity: implications for vaccine responsiveness. Expert Rev Vaccines **2008**; 7:467–79.
24. United States Department of Agriculture. USDA issues license for West Nile Virus DNA vaccine for horses. http://www.aphis.usda.gov/lpa/news/2005/07/wnvdna_vs.html. Accessed 1 October 2010.
25. Salazar P, Traub-Dargatz JL, Morley PS, et al. Outcome of equids with clinical signs of West Nile virus infection and factors associated with death. J Am Vet Med Assoc **2004**; 225:267–74.
26. Davis BS, Chang GJ, Cropp B, et al. West Nile virus recombinant DNA vaccine protects mouse and horse from virus challenge and expresses in vitro a noninfectious recombinant antigen that can be used in enzyme-linked immunosorbent assays. J Virol **2001**; 75:4040–7.
27. Spohn G, Jennings GT, Martina BE, et al. A VLP-based vaccine targeting domain III of the West Nile virus E protein protects from lethal infection in mice. Virol J **2010**; 7:146.
28. Martin JE, Pierson TC, Hubka S, et al. A West Nile virus DNA vaccine induces neutralizing antibody in healthy adults during a phase 1 clinical trial. J Infect Dis **2007**; 196:1732–40.
29. Barouch DH, Yang ZY, Kong WP, et al. A human T-cell leukemia virus type 1 regulatory element enhances the immunogenicity of human immunodeficiency virus type 1 DNA vaccines in mice and nonhuman primates. J Virol **2005**; 79:8828–34.
30. Catanzaro AT, Roederer M, Koup RA, et al. Phase I clinical evaluation of a six-plasmid multiclade HIV-1 DNA candidate vaccine. Vaccine **2007**; 25:4085–92.
31. Martin JE, Sullivan NJ, Enama ME, et al. A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial. Clin Vaccine Immunol **2006**; 13:1267–77.
32. Kibuuka H, Kimutai R, Maboko L, et al. A phase 1/2 study of a multiclade HIV-1 DNA plasmid prime and recombinant adenovirus serotype 5 boost vaccine in HIV-uninfected East Africans (RV 172). J Infect Dis **2010**; 201:600–7.
33. Pierson TC, Sanchez MD, Puffer BA, et al. A rapid and quantitative assay for measuring antibody-mediated neutralization of West Nile virus infection. Virology **2006**; 346:53–65.
34. Davis CW, Nguyen HY, Hanna SL, Sanchez MD, Doms RW, Pierson TC. West Nile virus discriminates between DC-SIGN and DC-SIGNR for cellular attachment and infection. J Virol **2006**; 80:1290–301.
35. Graham BS, Koup RA, Roederer M, et al. Phase 1 safety and immunogenicity evaluation of a multiclade HIV-1 DNA candidate vaccine. J Infect Dis **2006**; 194:1650–60.
36. Catanzaro AT, Koup RA, Roederer M, et al. Phase 1 safety and immunogenicity evaluation of a multiclade HIV-1 candidate vaccine delivered by a replication-defective recombinant adenovirus vector. J Infect Dis **2006**; 194:1638–49.
37. Martin JE, Louder MK, Holman LA, et al. A SARS DNA vaccine induces neutralizing antibody and cellular immune responses in healthy adults in a phase I clinical trial. Vaccine **2008**; 26:6338–43.
38. Martin JE, Pierson TC, Graham BS. Reply to Rottinghaus and Poland. J Infect Dis **2008**; 197:1628–9.
39. McElhaney JE. The unmet need in the elderly: designing new influenza vaccines for older adults. Vaccine **2005**; 23(Suppl 1):S10–25.
40. Giudice EL, Campbell JD. Needle-free vaccine delivery. Adv Drug Deliv Rev **2006**; 58:68–89.
41. Holland D, Booy R, De Looze F, et al. Intradermal influenza vaccine administered using a new microinjection system produces superior immunogenicity in elderly adults: a randomized controlled trial. J Infect Dis **2008**; 198:650–8.